Supplementary Information for

The ELF4-ELF3-LUX Complex Links the Circadian Clock to Diurnal Control of Hypocotyl Growth.

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Supplemental Table S1:

Supplemental Table **1:** sequence of primers used in this study

Primers used to generate yeast one-hybrid constructs (position relative to the transcriptional start site)

Amplified fragment	Forward primer (5' -> 3')	Reverse primer (5' -> 3')
PIF5 -813/-406	CACCCGGTAGGGGTTATGCACATTAAACGA	AGAGTCTCATTAAAACCCGAGCGGA

PIF5 -480/+13	CACCGCGATGTGGAAATTTGCCGGGG	AGCGAGTGAGCGAGGGGGAG
PIF5 +13 mutation (lowercase indicates mutated region, these primers are used with the -480/+13 primers to generate the mutated sequence)	CAGTACTATTGCCCCCACatccga-ATTATCTCCCCCTCGCTCACTCGCT	AGCGAGTGAGCGAGGGGGAGATAAT -tcggatGTGGGGGCAATAGTACT
PIF4 -894/-458	CACCGAAATCCGTATGGTCAAAATTATATTT	CGTTTATCACGTTGCATTGAA
PIF4 -474/-1	CACCATGCAACGTGATAAACGCC	ACAGGAGCATAAAGATATTACAGCG
PIF4 -1/+580	CACCCACTTTCTGTCTGTACCCAAAAGA	CATGTCAGATCTCTGGAGACATTT

Primers used for ChIP assays (position relative to transcriptional start site)

Amplicon	Forward primer (5' -> 3')	Reverse primer (5' -> 3')
UBQ -253/-32	AATAAACGGCGTCAAAGTGG	ACGAGGACGACTAGGTCACG
PIF5 (5A) -845/-708	CGGTAGGGGTTATGCACATTAAACGA	TGGACCACAGACAAACAATGGTCCAC
PIF5 (5B) -481/-406	GCGATGTGGAAATTTGCCGGGG	AGAGTCTCATTAAAACCCGAGCGGA
PIF5 (5C) -269/-146	CGCTGAAAGAGAAGCATAAGAGGGGT	TGCTCGATTTCTAGAGGTGGGTTTCT
PIF5 (5D) -117/+13	TCCAACCGAGTTGGTGGGTCTCA	AGCGAGTGAGCGAGGGGGAG
PIF5 CS +1986/+2110	CCGCTCCGCAGAACCATCCC	CCGGCGGCATCTGGGGAATC
PIF4 (4A) -501/-414	GCCAAGGGTGCCCTTTCAATGC	CCGAGTTCAATGCTCTCAACGAGT
PIF4 (4B) -10/+152	TGCTCCTGTCACTTTCTGTCTGTACCC	TCCAAGTTCCACGCCCAACACA
PIF4 (4C) +194/+272	TCTGATTCGTCCAGAAGCTTTCCT	GCCACATCTTATAAAACCAAAAACCCG
PIF4 (4D) +581/+735	ACACCAAGGTTGGAGTTTTGAGGA	TGGCCTAGACATCAATACACACACACA
PIF4 CS +1980/+2150	TCCCCGGAGTTCAACCTCAGCA	GAGTCGCGGCCTGCATGTGT

Primers used for expression analysis by qRT-PCR

Gene	Forward primer (5' -> 3')	Reverse primer (5' -> 3')

IPP2	GTATGAGTTGCTTCTGGAGCAAAG	GAGGATGGCTGCAACAAGTGT
APX3	GCCGTGAGCTCCGTTCTCT	TCGTGCCATGCCAATCG
At1g11910	CTCCAGAAGAGTATGTTCTGAAAG	TCCCAAGATCCAGAGAGGTC
ELF3	GCACAGACTGATTAAGGTTCAAAAAC	CTTCACTGGATAGCTTTTAGCAG
LUX	TAACGTGGAGGAGGAAGATCGA	TCCATCACCGTTTGATGTCTTT
ELF4	TGTCGTTGACTTGTTGAATCAGTG	CGATGTGGGAGAATCTTGAC
PIF4	GTTGTTGACTTTGCTGTCCCGC	CGACTCAGCCGATGGAGATGTT
PIF5	CGCCGGAGATCCAAATCCCAACAT	GCGGGAAATCAGACCGTGCAACAA
NOX	TGCTGAAAGCTAACGCGAGAA	TGGTTTACCCAACGGAGATGA

Primers used for amplification of full length cDNAs or fragments not described in materials and methods.

Gene	Forward primer (5' -> 3')	Reverse primer (5' -> 3')
ELF3	CACCATGAAGAGAGGGAAAGAT	TTAAGGCTTAGAGGAGTC
ELF4	CACCATGAAGAGGAACGGCGAGACGA	AGCTCTAGTTCCGGCAGCACC
LUX-N	CACCATGGGAGAGGAAGTACAAA	TTTAAGTGTTTTCCCAGATAG
LUX-C	CACCCGACCGCGTTTAGTGTGG	ATTCTCATTTGCGCTTCC

Supplemental Figure Legends:

Figure S1) **Diurnal and circadian time course data for *LUX*, *ELF3* and *ELF4* from DIURNAL**. Expression profiles for *LUX* (top), *ELF3* (middle) and *ELF4* (bottom) grown in diurnal (shortday = 8h:16h light:dark (L:D), 22 ˚C; longday =16h:8h L:D 22 ˚C; LLHC = constant light, 12h 22 ˚C:12h 12 ˚C; LDHC = 12h:12h L:D, 12h 22 ˚C:12h 12 ˚C; LDHH_SM = 12h:12h, L:D, 20 ˚C) or circadian (LL_LLHC = release into constant light and temperature after growth in LLHC; LL_LDHC = release into constant light and temperature after growth in LDHC; LL23_LDHH = measured starting on the second day after release into constant light and temperature constant light after growth in 12h12h L:D, 22 ˚C; DD_DDHC = release into constant darkness and temperature after growth in constant darkness, 12h 22 ˚C:12h 12 ˚C). gcRMA refers to GeneChip Robust Multiarray Averaging of values. Full details can be found at the DIURNAL website (http://diurnal.cgrb.oregonstate.edu/)[18,19]).

Figure S2) ***ELF4*::ELF4-HA rescues hypocotyl length and rhythmicity of the *elf4-2*.** a) Promoter driven ELF4-HA lines rescue hypocotyl length of 10-day-old seedlings grown in 12h:12h light:dark conditions. n=20 left graph, n=15 right graph. This experiment was repeated twice with similar results. b) Bioluminescence analysis of *CAB2::LUC* in Col-0, *ELF4*::ELF4-HA *elf4-2* #1, *ELF4*::ELF4-HA *elf4-2* #2, and *elf4-2* in constant light. The grey vertical bars in the background of the graph denote the subjective evening. Traces are the mean value for each time point, with the error bars representing S.E.M, n=24. This experiment was repeated twice with similar results. c) Relative amplitude error (RAE) versus period plots of *CAB2::LUC* for Col-0, *ELF4*::ELF4-HA *elf4-2* #1, *ELF4*::ELF4-HA *elf4-2* #2, and *elf4-2*. The dotted line at 0.6 RAE represents a cutoff above which a seedling is not considered rhythmic. 91% of Col-0, 92% of *ELF4*::ELF4-HA *elf4-2* #1, 50% of *ELF4*::ELF4-HA *elf4-2* #2 were found to be rhythmic, but none of the *elf4-2* seedlings were (Col-0 n= 22, *ELF4*::ELF4-HA *elf4-2* #1 n=24, *ELF4*::ELF4-HA *elf4-2* #2 n=24 and *elf4-2* n=24.) d) Scatter plots for calculated periods for Col-0 (23.75 ± 0.11 hours, n=22), *ELF4*::ELF4-HA *elf4-2* #1 (21.5 ± 0.13 hours, n=24) and *ELF4*::ELF4-HA *elf4-2* #2 (22.01 ± 0.13 hours, n=20) ± values represent S.E.M. e) Comparison of *ELF4* expression in wild type versus *ELF4*::ELF4-HA *elf4-2* #1 from a diurnal to constant light time course (top) (the graph is a combination data presented in Figure 1a and S4) and *ELF4*::ELF4-HA *elf4-2* #1 versus *ELF4*::ELF4-HA *elf4-2* #2 at ZT12 (bottom) (all lines *CAB2*::LUC). Expression was normalized to *IPP2, APX3* and *At1g11910,* and the error bars represent the S.E.M. from 3 independent time courses/samples. f) Both *ELF4*::ELF4-HA lines co-immunoprecipitate ELF3 and LUX. HA-epitope immunoprecipitations (IP) were performed in control Col-0 (containing a *CAB2::LUC* reporter), *ELF4*::ELF4-HA *elf4-2 CAB2::LUC* #1, *ELF4*::ELF4-HA *elf4-2 CAB2::LUC*

#2, *CCA1::LUC* or *35S*::GFP-HA *CCA1::LUC* background, harvested on day 12 at ZT12, 12h:12h light:dark growth cycles. Western blots using affinity-purified ELF3 and LUX antibodies detected endogenous ELF3 and LUX, and anti-HA was used to detect ELF4-HA or GFP-HA. Blots for ELF4 and GFP represent 20% of the total IP sample, as ELF4 and GFP must be run on a separate 15% gel because of their low molecular weight; these gels are noted by an asterisk (*). The dot (•) denotes a background signal arising from the cross-linked HA beads (data not shown). LUX runs as high and low molecular weight isoforms, which are lost in the *lux* background, and are denoted by (–). ACTIN serves as a control for loading. These experiments were performed three times on independent samples with similar results.

Figure S3) **Comparison of *lux-4, elf4-2, elf4-3, elf3-1, elf3-1 lux-4 and elf3-4 elf4-3* hypocotyl lengths.** a) Hypocotyl measurements of wild type, *lux-4, elf4-2, elf4-3, elf3-1, elf3-1 lux-4 and elf3-4 elf4-3* mutants were made on 10-day-old seedlings grown in 12h:12h light:dark conditions, n=15. Wild type, *elf4-2, elf4-3, lux-4, elf3-1 lux-4 and elf3-1 elf4-3* contain *CAB2::LUC* reporters. This experiment was repeated twice with similar results. b) Characterization of the *elf4-3* circadian phenotype. Bioluminescence analysis of *CAB2::LUC* in Col-0 and *elf4-3* in constant light. The grey vertical bars in the background of the graph denote the subjective evening. Traces are the mean, with the error bars representing S.E.M, n=24. This experiment was repeated twice with similar results. c) Relative amplitude error (RAE) versus period plots for Col-0 and *elf4-3*. The dotted line at 0.6 RAE represents a cutoff above which a seedling is not considered rhythmic. One of the *elf4-3* seedlings was considered rhythmic versus 20 for Col-0 (Col-0 n= 22 and *elf4-3* n=24.)

Figure S4) **Expression profiles of *ELF4*, *ELF3* and *LUX* in the *ELF4::*ELF4-HA *elf4-2* lines.**
Expression of *ELF4, ELF3* and *LUX* in the *ELF4*::ELF4-HA *elf4-2* #1 background under diurnal to constant light conditions (top), long day (middle) or short day conditions (bottom). mRNA levels are normalized to *IPP2, APX3,* and At1g11910 and each transcript is normalized relative to its maximum expression. The bars above the graphs represent light conditions during harvesting; black = lights off, white = lights on, grey = lights on during subjective night. Error bars represent the S.E.M. of the average of duplicate measurements from 3 independent time courses.

Figure S5) **ELF3 and LUX levels in diurnal or circadian conditions in Col-0.**
A representative western blot of Col-0 seedlings harvested every four hours beginning at ZT12 in the light on day 12. Seedlings were transferred to constant light conditions on the morning of day 13, just after harvesting the ZT0 time point in the dark. Westerns blots using affinity-purified ELF3 or LUX antibodies detected endogenous ELF3 and LUX, and ACTIN antibodies serve as a control for loading. LUX runs as high and low molecular weight isoform, which are lost in the *lux* background, and are denoted by (–). Expression time courses are re-plotted from Figure 1A for comparison. These experiments were repeated three times with similar results.

Figure S6) **Comparison of the Evening Complex and *PIF4* and *PIF5*.**
Expression of *PIF4* and *PIF5* in the ELF4::ELF4-HA #1 *elf4-2* background under diurnal to constant light conditions, long and short days compared to EC levels. The *PIF4* and *PIF5*

expression profiles are aligned to the corresponding time points in the western blot of the EC. RNA expression levels are normalized to *IPP2, APX3* and *At1g11910* and then each transcript is normalized relative to its maximum expression. The bars above the blots represent light conditions during harvesting; black = lights off, white = lights on, grey = lights on (subjective night). Error bars represent the S.E.M. from 3 independent time courses. These data include the data from Figures 2b, c and d.

Figure S7) **Expression of *PIF4* and *PIF5* is elevated in LUX-VP64.**

Expression of *PIF4* and *PIF5* in wild type, *lux-4* or *35S*::LUX-VP64 background at ZT16. Expression levels are normalized to *IPP2, APX3* and *At1g11910.* The data reflect the average of two independent replicates measured twice. Error bars represent the standard deviation from the mean.

Figure S8) **ELF3 enrichment at the *PIF4* and *PIF5* promoters is greater near the peak of ELF3 levels.** Col/*elf3-1* ChIP enrichment ratios at the *PIF4* and *PIF5* promoters with the ELF3 antibody were measured at ZT2 versus ZT14. *UBIQUITIN* promoter values were plotted on both graphs for comparison. Error bars represent the S.E.M, n=3.

Figure S9) **ELF3 ChIP in the *lux-4* background**

Endogenous ELF3 ChIP on *PIF5* and *PIF4* promoters at ZT14 from either *elf3-1* control, *lux-4* (*CAB2*::LUC) or wild-type (*CAB2::LUC*) lines. The data reflect the average of two technical replicates measured twice. Error bars represent the standard deviation from the mean. This experiment was repeated with similar results.

Figure S10) **Analysis of the LUX/NOX ami lines.** a) NOX interacts with ELF3 and ELF4 in a yeast three-hybrid assay. Yeast containing combinations of ELF4-GAL4-DBD, NOX-GAL4-AD and ELF3 were assayed for activation of the LacZ reporter. Error is represented as S.E.M. of 3 technical repeat measurements from 4 independent transformants, normalized to empty bait and prey vector controls. b) Expression of *NOX* in wild type, *lux-4* or LUX/NOX ami (all *CAB2*::LUC) background at ZT4, ZT12 or ZT16. Expression levels are normalized to *IPP2, APX3* and *At1g11910*. The data reflect the average of two independent replicates measured twice. Error bars represent the standard deviation from the mean. c) ELF3 and LUX levels in wild type, *elf3, lux-4* or the LUX/NOX ami lines at ZT12. ACTIN serves as a control for loading. LUX runs as high and low molecular weight isoforms, which are lost in the *lux* background, and are denoted by (–). This experiment was repeated with similar results. d) Hypocotyl measurements of wild-type, *elf3-1, lux-4,* and LUX/NOX ami lines were taken on 10-day-old seedlings grown in 12h:12h light:dark conditions, n=15. Wild-type, *lux-4,* and LUX/NOX ami lines contain *CAB2::LUC* reporters. This experiment was repeated twice with similar results. e) Characterization of the circadian phenotype of LUX/NOX ami. Bioluminescence analysis of *CAB2::LUC* in wild type (Col-0) and LUX/NOX ami in constant light. Traces are the mean, with the error bars representing S.E.M, n=20. The grey vertical bars in the background of the graph denote the subjective evening. This experiment was repeated twice with similar results. f) Relative amplitude error (RAE) versus period plots for wild type and LUX/NOX ami. The dotted line at 0.6 RAE represents a cutoff above which a seedling is not considered rhythmic. Seven of the LUX/NOX ami seedlings were considered rhythmic versus 20 of Col-0 (n=20 for each). g) Expression of

PIF4 and *PIF5* in wild type, *lux-4* or LUX/NOX ami background at ZT4, ZT12 or ZT16. Expression levels are normalized to *IPP2, APX3* and *At1g11910* and the error bars represent the SD from 2 independent samples measured twice.

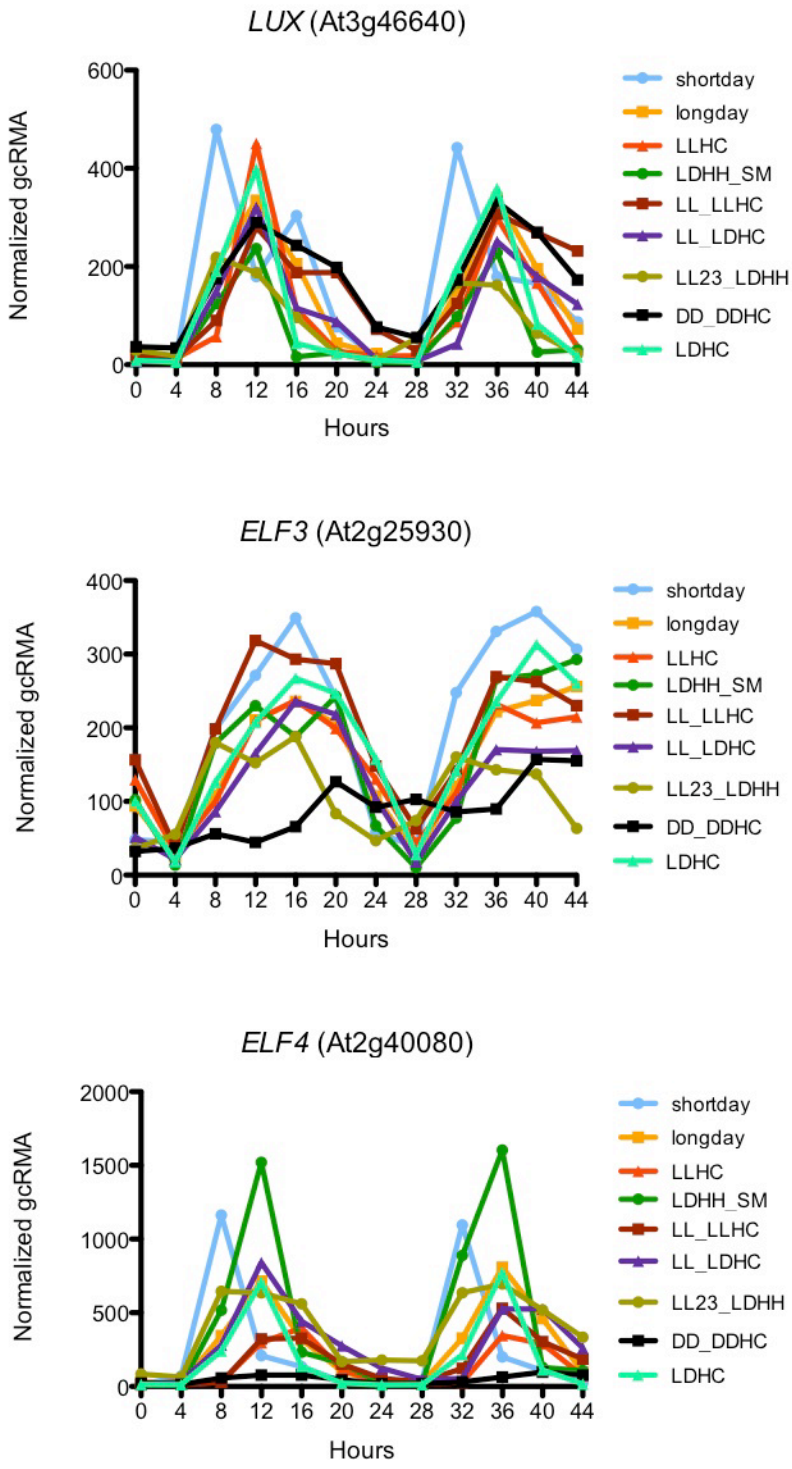
Figure S11) ***PIF5, PIF4, ELF4, LUX* and *ELF3* expression patterns in *lhy-1*.**

Expression profiles for *PIF5, PIF4, ELF4, LUX*, and *ELF3* in either wild type (Lansberg erecta) or *lhy-1* background in short-day (8h:16h light:dark (L:D), 22 ˚C) conditions from the DIURNAL website. Night is denoted by the black bars in the background of the graph. gcRMA refers to GeneChip Robust Multiarray Averaging of values. Full details can be found at the DIURNAL website (http://diurnal.cgrb.oregonstate.edu/)[18,19].

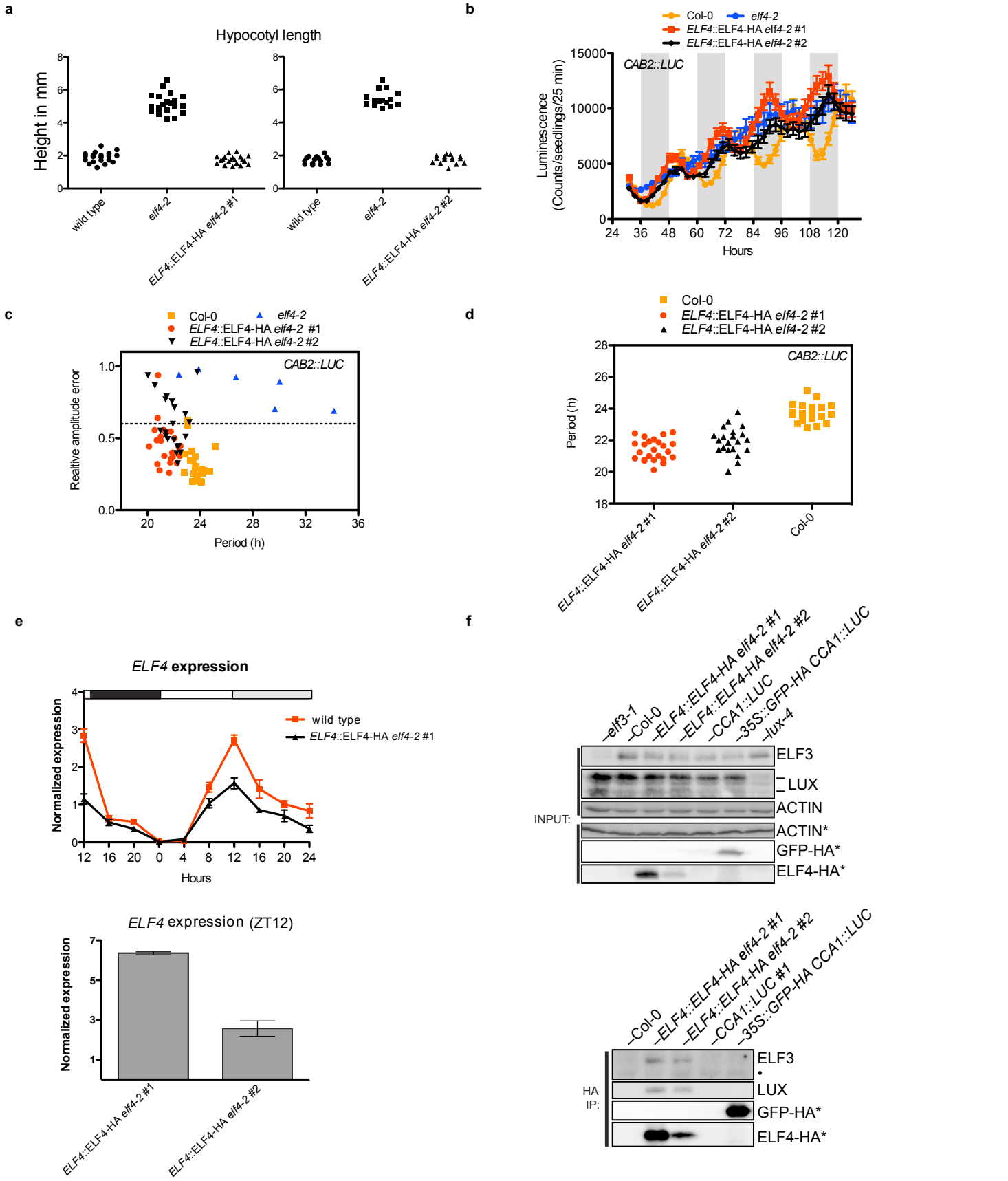
Figure S12) ***pif4* and/or *pif5* do not feedback into the clock or rescue circadian rhythms in the *elf3* background.** a) Bioluminescence analysis of *TOC1::LUC* in *elf3-2 pif4-101 pif5-1* mutants in constant light. Traces are the mean, with the error bars representing S.E.M, n=12. The grey vertical bars in the background of the graph denote the subjective evening. This experiment was repeated twice with similar results. b) Relative amplitude error (R.A.E.) versus period plots of the *TOC1::LUC* reporter in *elf3-2 pif4-101 pif5-1* mutants. The dotted line at 0.6 R.A.E. represents a cutoff above which is not considered rhythmic. 100% of the Col-0 and *pif4-101* and/or *pif5-1* were found to be rhythmic, but only a single line from the *elf3-2* genotype and two lines from the *elf3-2 pif4 pif5* genotypes were determined to be rhythmic. This experiment was repeated twice with similar results. c) Scatter plots for calculated periods for Col-0 (24.00 ± 0.08 hours, n=12), *pif5* (23.90 ±

0.09 hours, n=10), *pif4* (24.00 ± 0.07 hours, n=10), and *pif4 pif5* (23.74 ± 0.08 hours, n=12). The error bars represent ± S.E.M. This experiment was repeated twice with similar results.

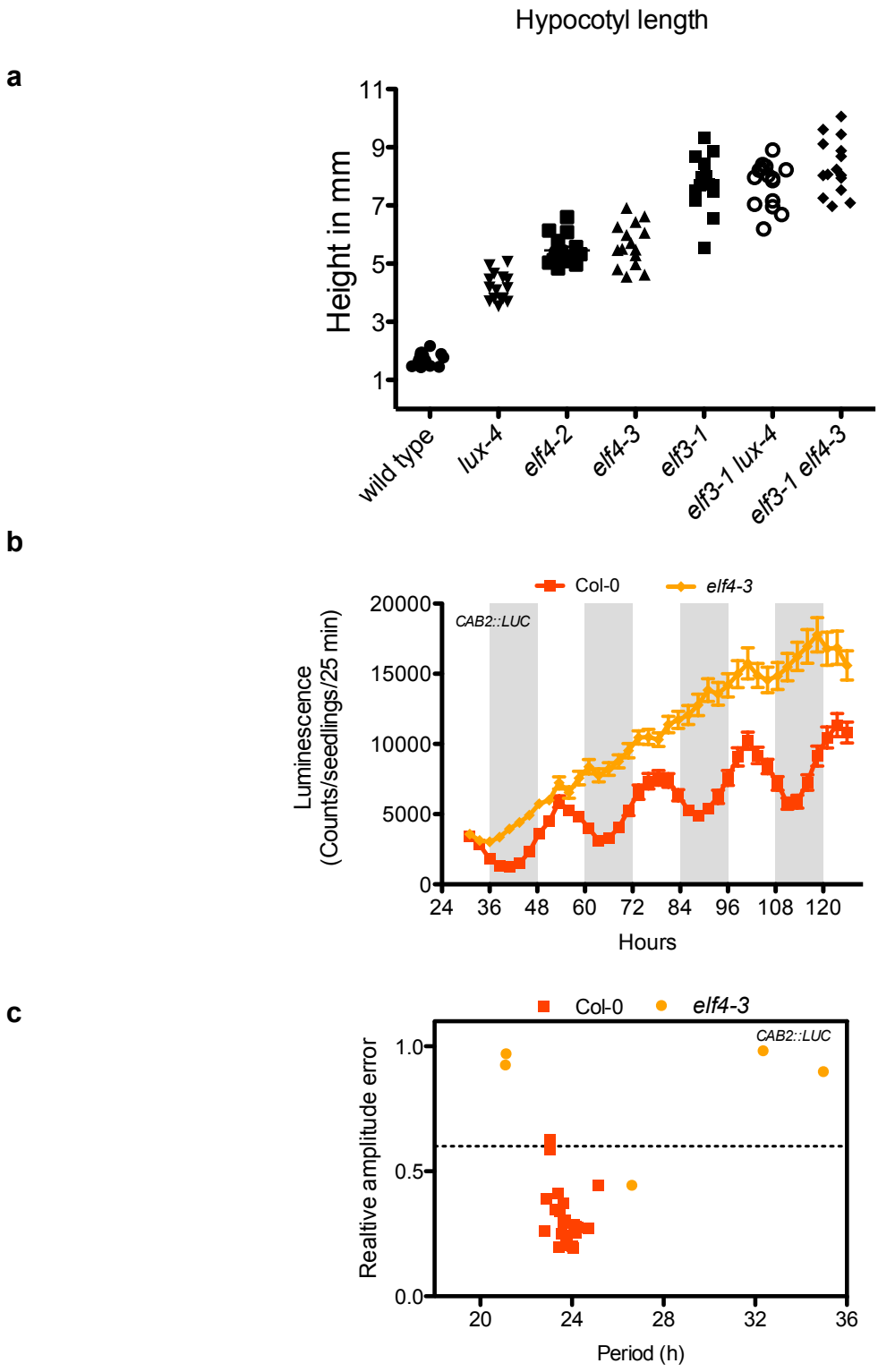
Supplemental Figure 1



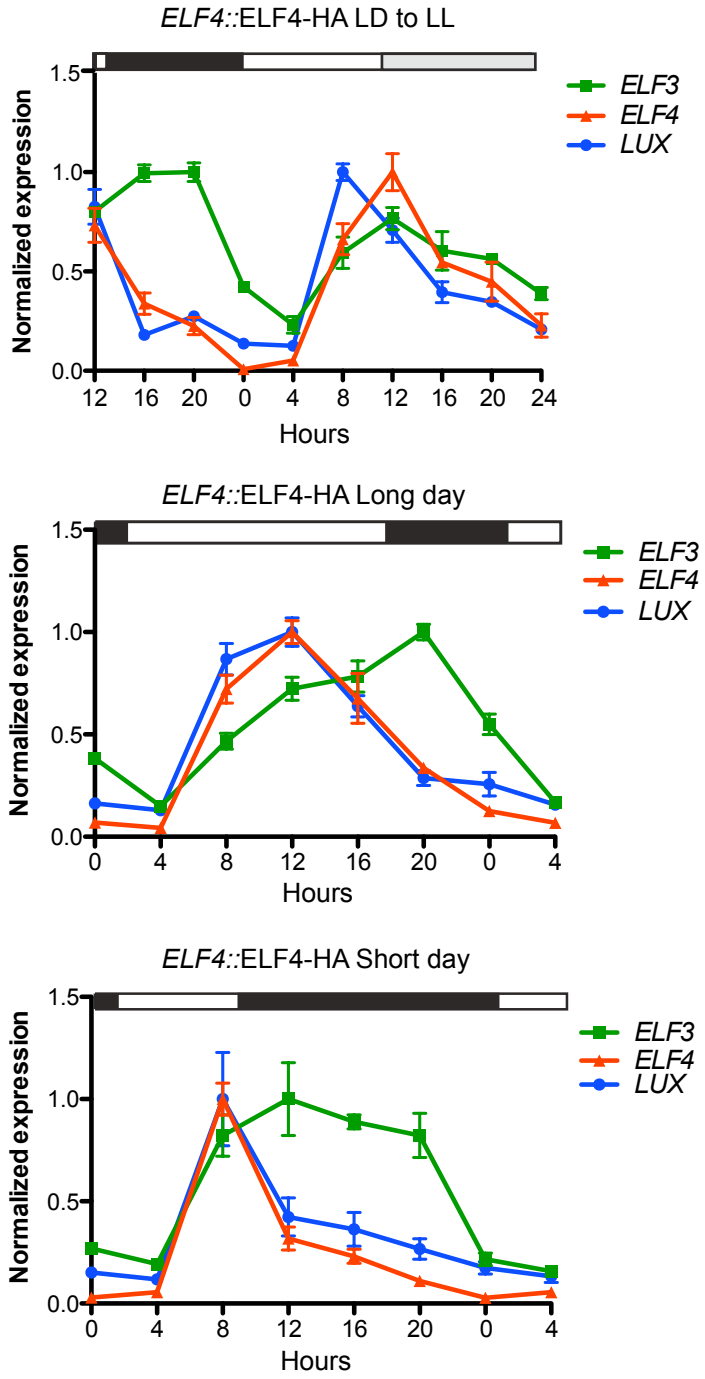
Supplemental Figure 2



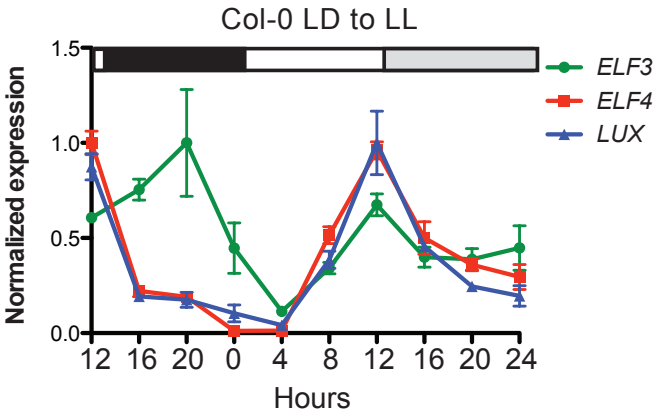
Supplemental Figure 3

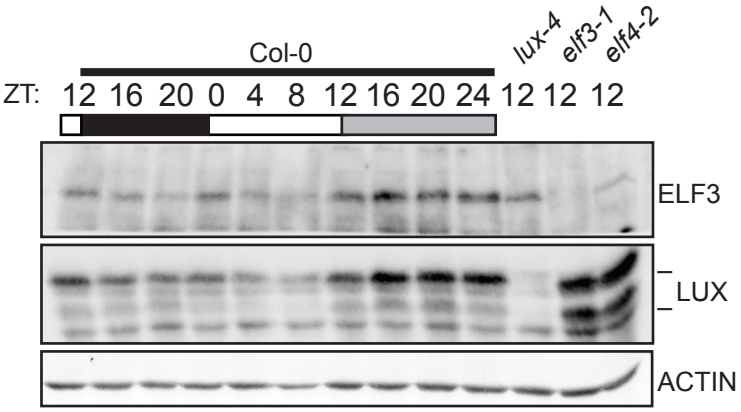


Supplemental Figure 4

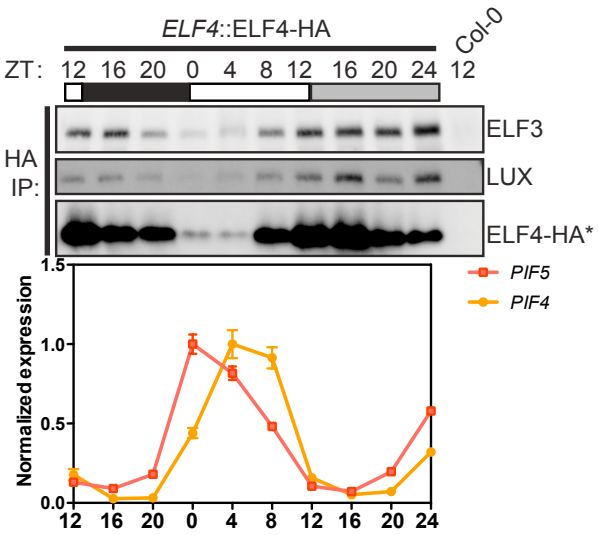


Supplemental Figure 5

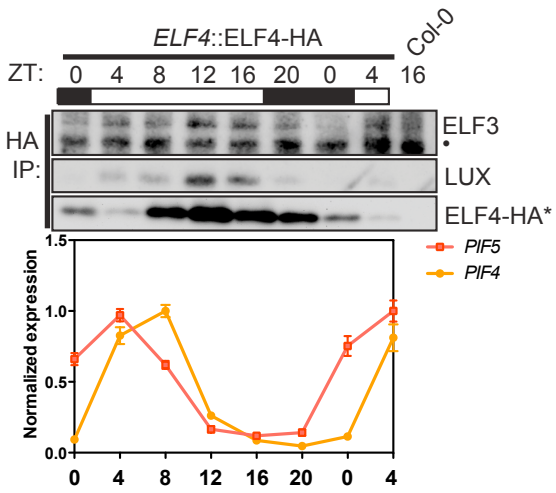




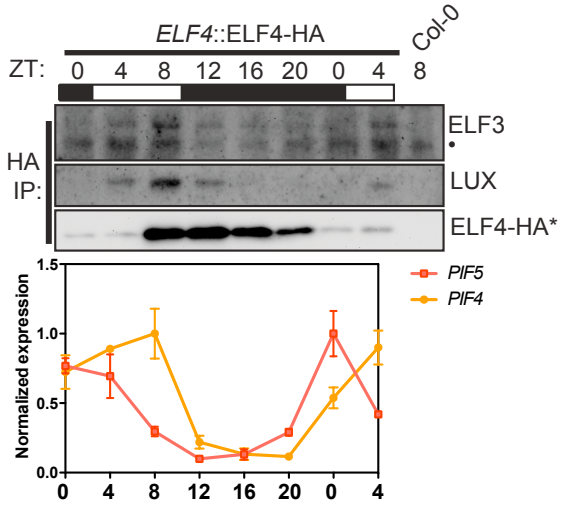
Supplemental Figure 6



ELF4::ELF4-HA LD to LL

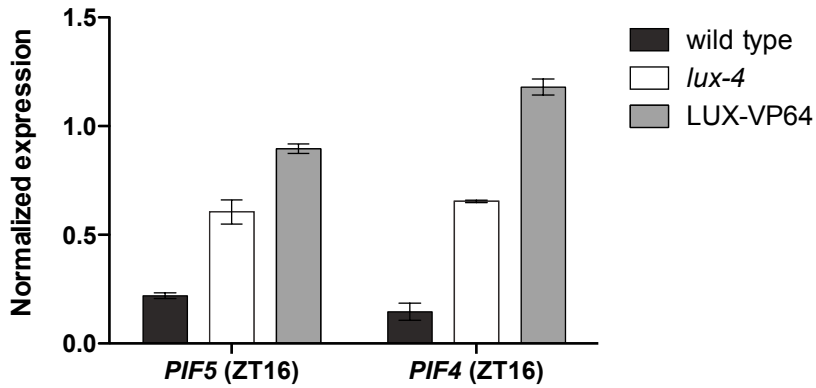


ELF4::ELF4-HA Long day

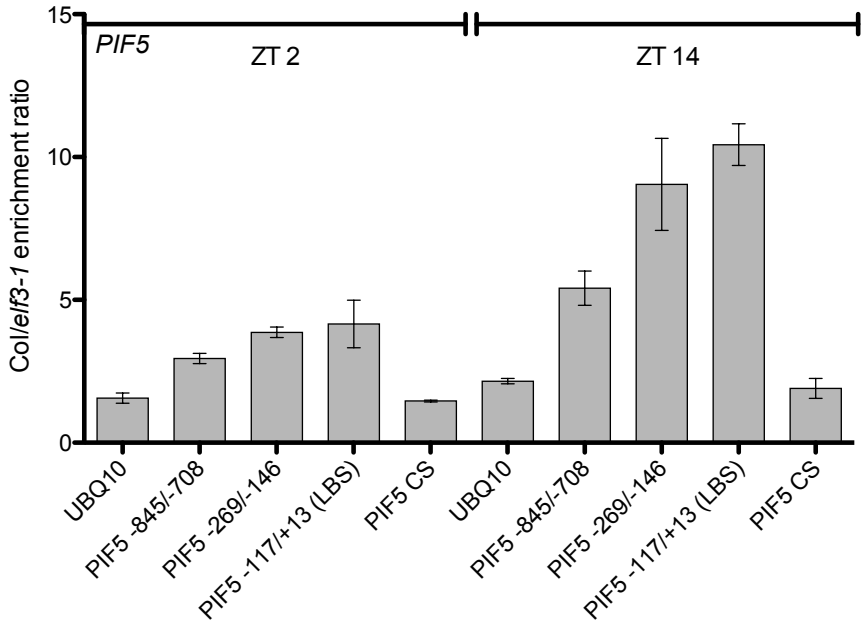


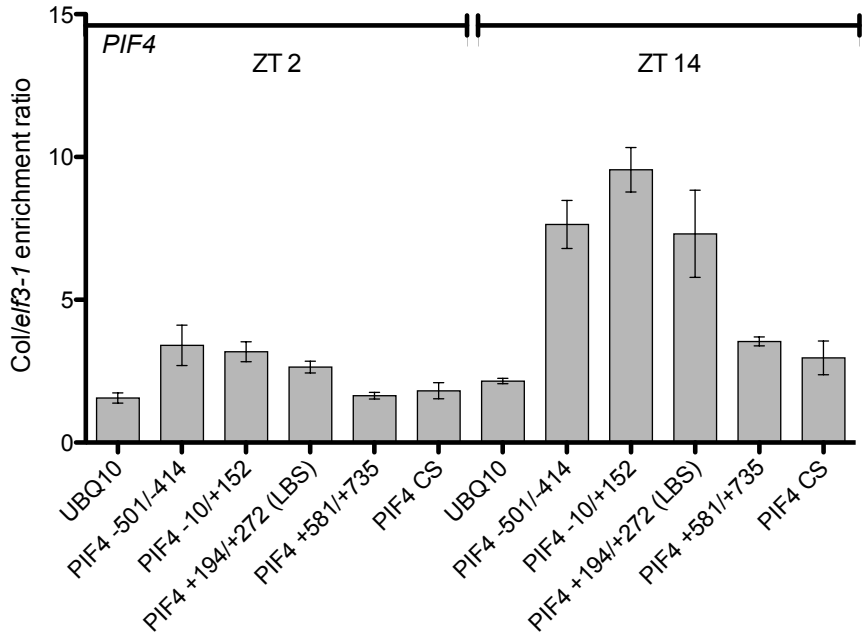
ELF4::ELF4-HA Short day

Supplementary Figure 7

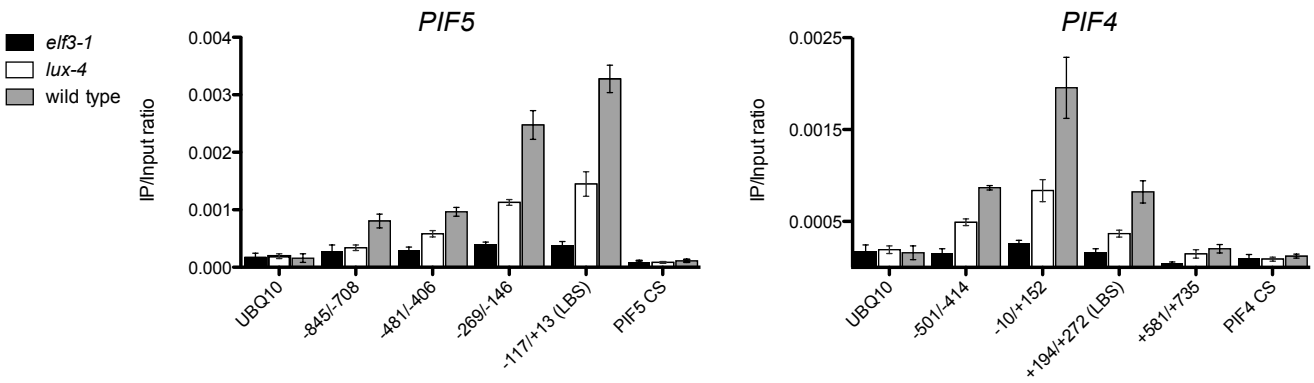


Supplemental Figure 8

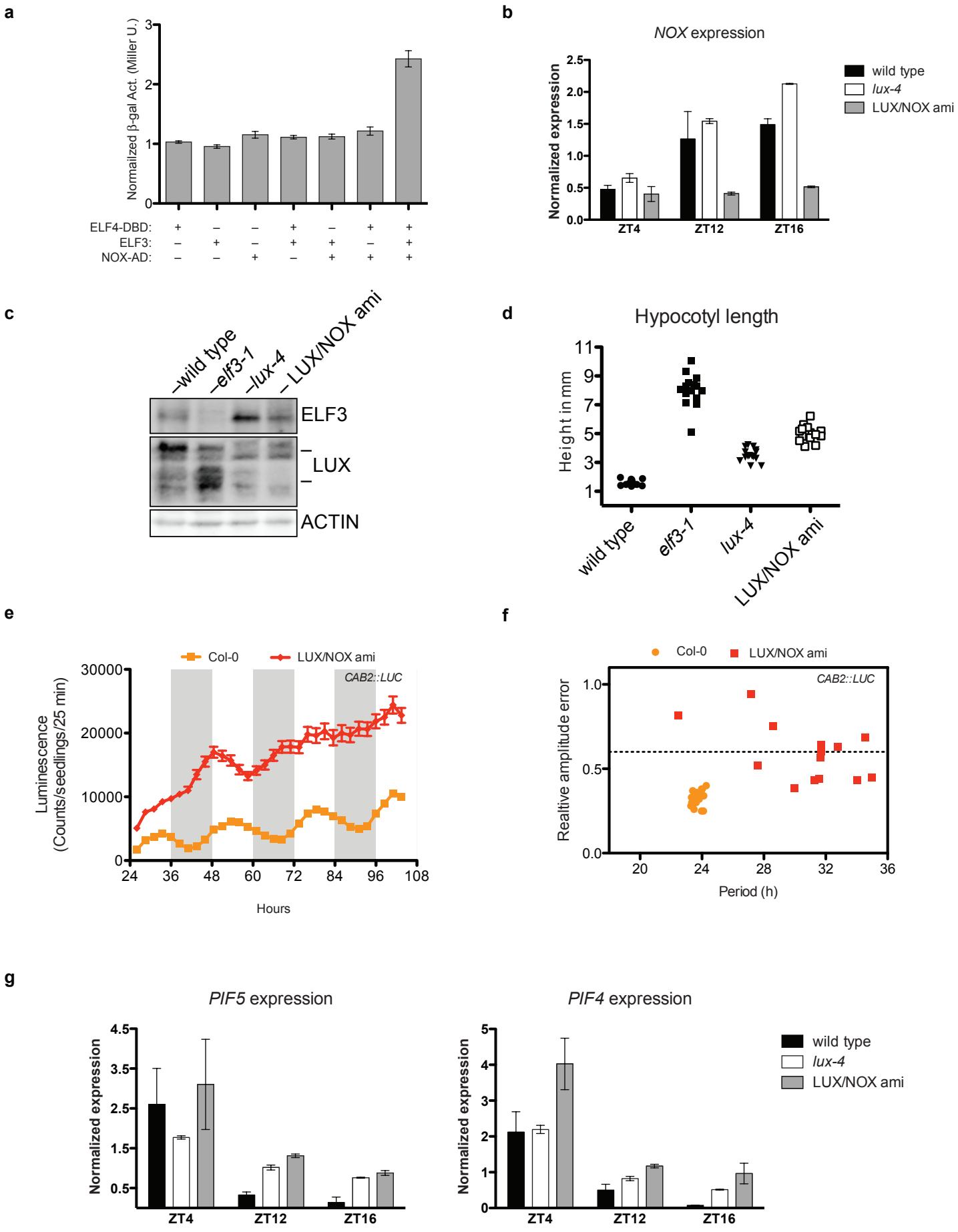




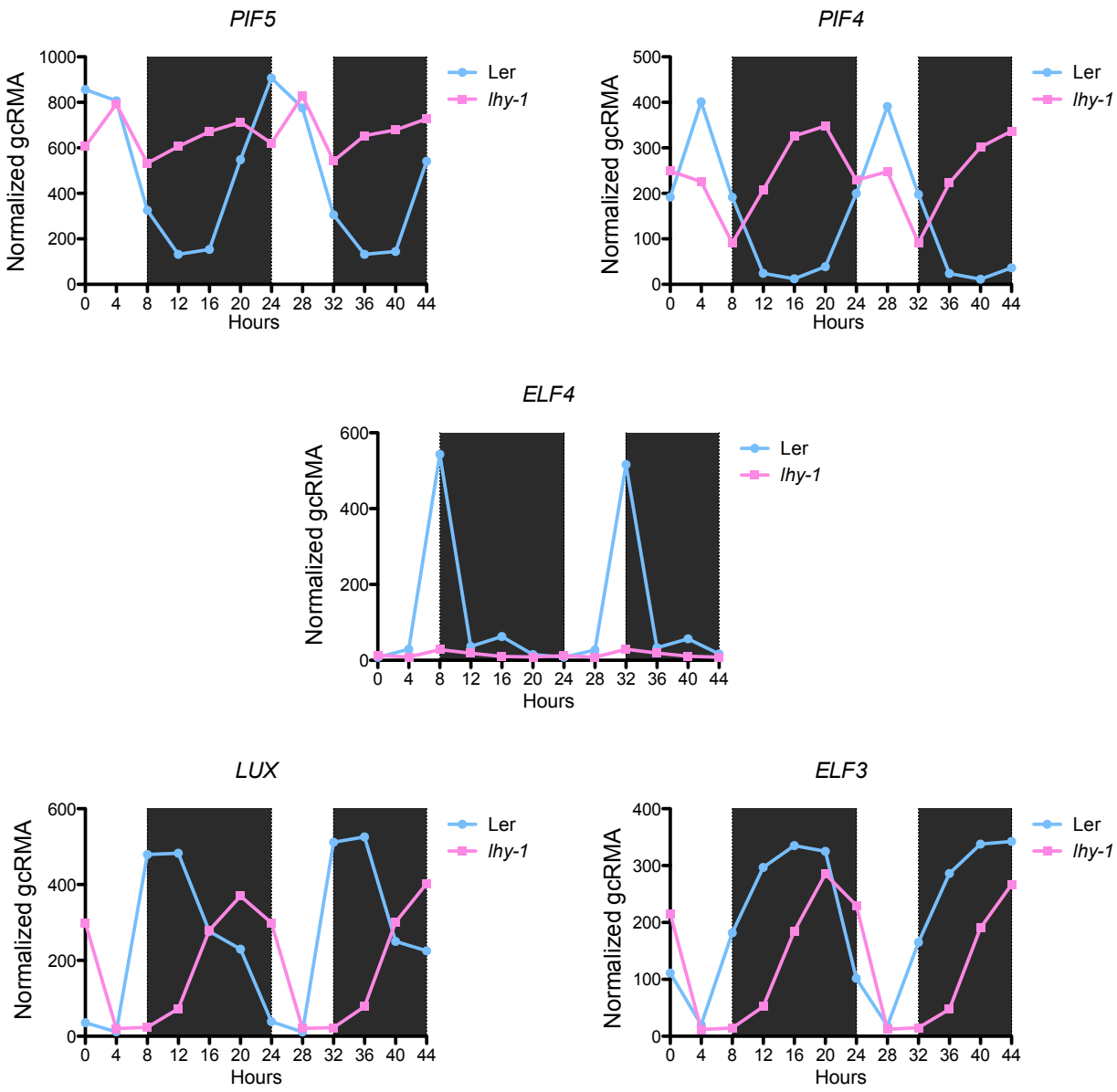
Supplemental figure 9



Supplemental Figure 10



Supplemental Figure 11.



Supplemental Figure 12